The Furan Shuffling Hypothesis: A Biogenetic Proposal for Eremophilane Sesquiterpenoids

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The Furan Shuffling Hypothesis: a Biogenetic Proposal for Eremophilane Sesquiterpenoids

*Nicolas Lardon,[‡] Raphael Liffert,[‡] Anthony Linden, and Karl Gademann**

Dedication ((optional))

Abstract: *Based on the structural similarities of the recently isolated eremophilane-type sesquiterpenoids microsphaeropsisin B and C and the iso-eremophilane periconianone C, we suggest a revised biogenetic hypothesis for C8–C11 connected iso-eremophilanes. In this communication, we provide strong experimental evidence corroborating our biogenetic proposal. The first enantioselective total syntheses of microsphaeropsisin B and C have been achieved starting from a known intermediate, whose synthesis has been elaborated in the course of the total synthesis of periconianone A in our group, and in a total of 15 steps starting from γ-hydroxy carvone. Mild reaction conditions for the subsequent α-ketol rearrangement not only allowed for our proposed conversion of microsphaeropsisin B to periconianone C, but also for the transition of microsphaeropsisin C to 4-epi-periconianone C.*

Eremophilanes belong to the family of sesquiterpene natural products and are structurally characterized by a decalin core bearing two one-carbon residues at C-4 and C-5, and a three-carbon moiety (isopropyl or isopropenyl) at C-7.[1] As their constitution cannot be rationalized by Ružička's *Isoprene Rule*,[2] the structural elucidation of the first isolated member named eremophilone was challenging at the time. Robinson unraveled the mystery, when he suggested a biosynthetic pathway from an eudesmane precursor by methyl migration from C-10 to C-5 to form this new skeleton.[3] Although we are aware of methyl migrations and carbon skeleton rearrangements in terpene biosynthesis today, his suggestions were of startling novelty at the time and can be considered a milestone for subsequent investigations of terpene biosynthesis. Over the years, isolation and characterization of several eremophilane synthases, as well as investigation of their mechanisms of action, substantiated Robinson's proposal that the biosynthesis of eremophilanes proceeds *via* an eudesmane cation.[4] Most of the isolated and characterized eremophilanes are bicarbocyclic and around half of them feature an additional oxocycle (furane or lactone) that is fused across positions C-7 and C-8 to form the corresponding furanoeremophilanes or eremophilenolides.[5] One example of a furan-type eremophilane is microsphaeropsisin B (**1**), a member isolated from co-cultivation of the endophytic fungus *Trichoderma* sp. 307 with the aquatic pathogenic bacterium *Acinetobacter johnsonii B2* (Figure 1).[6] Recently, the group of Dai reported the isolation of periconianone C (**2**), an unusual furan-type *iso*-eremophilane that was also isolated from an endophytic fungus, namely *Periconia* sp. F-31.[7] The structure of periconianone C (**2**) was confirmed by single-crystal X-ray analysis, which revealed the three-carbon moiety to be positioned at C-8 of the decalin core and not at position C-7, as in all congeners previously described. The group of Dai suggested that this linkage originates biosynthetically from a pinacol rearrangement of the dihydroxylated eremophilane **3** (Figure 1). Additional oxidation events of rearranged compound **4** and ketalization would then form periconianone C. This biogenetic proposal is supported by the isolation of the putative precursor **3** from the same extracts of the fungus as **2**.

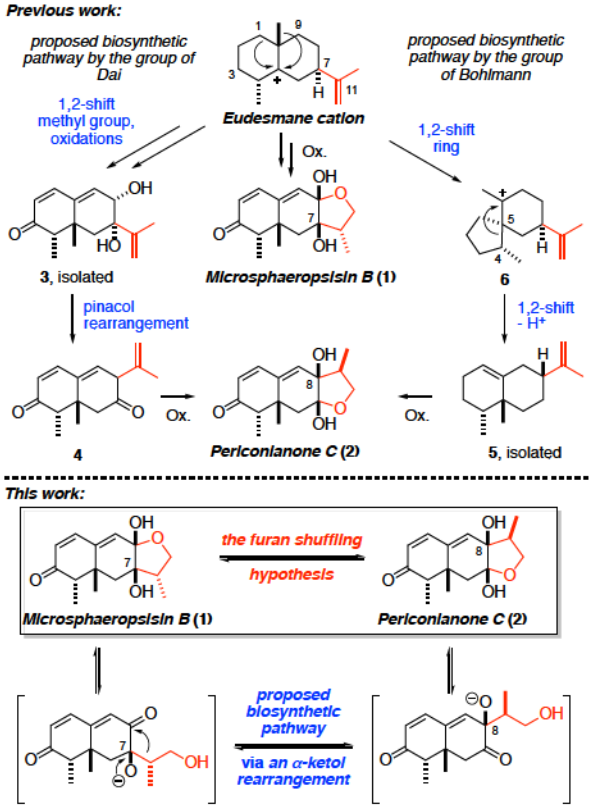

Figure 1. Proposed biosynthetic pathways as previously described, and our biosynthetic hypothesis with microsphaeropsisin B (**1**) as the precursor of periconianone C (**2**).

A different biosynthetic proposal to a C-8 propenylated *iso*-eremophilane was formulated by the group of Bohlmann after bicyclic diene **5** was isolated in small amounts from the roots of *Helichrysum davyi* S. Moore.[8] Bohlmann suggests that instead of methyl group migration in an eudesmane cation to form the eremophilane skeleton, the C-1–C-10 bond might undergo a 1,2-alkyl shift to form spiro intermediate **6**, whose C-4–C-5 bond can undergo another 1,2-shift to form hydrocarbon **5**. Subsequent oxidation events on **5** might then lead to the

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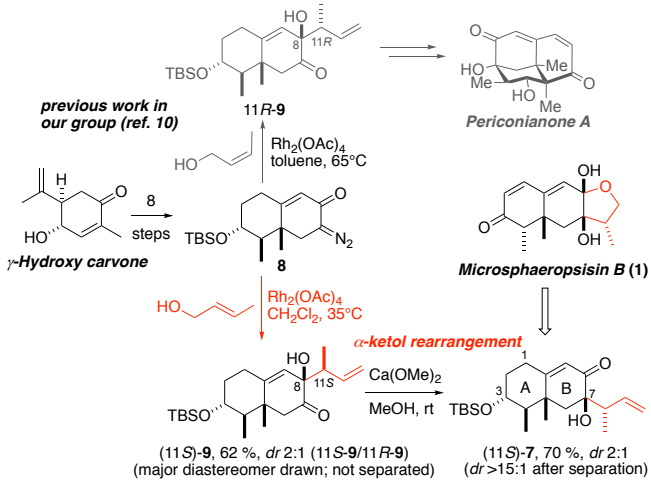
formation of periconianone C (**2**). However, a mechanism featuring an early rearrangement of the eudesmane cation *via* spiro intermediates was ruled out by labelling studies in the biosynthesis of capsidiol[9] and, as no other oxidized derivatives of C-8–C-11-connected eremophilanes have been described to date, this proposal seems less feasible.

In the course of the total synthesis of periconianone A recently accomplished in our group,[10] we discovered that bicyclic α-allylated α-hydroxyketones can undergo 1,2-shifts at positions C-7 and C-8 (Scheme 1). Based on these findings as well as the structural similarities of microsphaeropsisin B (**1**) and periconianone C (**2**), we herein suggest an alternative biogenesis for C-8–C-11-connected furanoeremophilanes by an equilibrium between the open forms of **1** and **2** *via* an α-ketol rearrangement (Figure 1). The mechanistic simplicity of this *furan shuffling hypothesis* paired with its feasibility both in nature and during isolation render this mechanistic rationale intriguing. In this work, we will provide strong evidence from total synthesis and spectroscopic investigations corroborating the furan shuffling hypothesis as a viable pathway in the biogenesis of these compounds.[11]

In order to investigate our biogenetic proposal, we developed an efficient synthetic route to microsphaeropsisin B (**1**). Starting from (11*S*)-α-alkylated α-hydroxyenone **7**, whose synthesis had been elaborated in the course of the synthesis of periconianone A,[10] we envisioned modifying the A-ring as well as the three-carbon side chain by a stepwise oxidation protocol to give access to microsphaeropsisin B. With furan **1** at hand, we planned to investigate experimentally the aforementioned transition of microsphaeropsisin B to periconianone C by an α-ketol rearrangement.

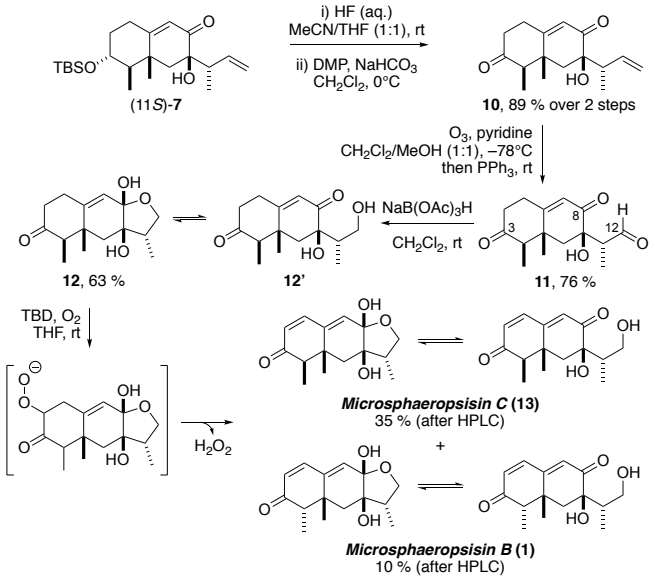
Our synthesis of microsphaeropsisin B (**1**) commenced from (11*S*)-**7**, which was synthesized from diazoketone **8** by a tandem O-H insertion/[3,3]-sigmatropic rearrangement and subsequent α-ketol rearrangement of the α-allylated α-hydroxyketone (11*S*)-**9**. The diazoketone itself was synthesized in eight steps from known γ-hydroxy carvone.[10]



Scheme 1. Preparation of precursor (11*S*)-**7** as the central intermediate in the total synthesis of microsphaeropsisin B (**1**).

The main challenge for the transition of **7** to microsphaeropsisin B (**1**) was to find a suitable sequence for achieving the desired oxidation states on the functionalized positions, *i.e.* to optimize the order in which these reactions were carried out on the A-ring and on the side chain. Oxidative cleavage of the terminal double bond in (11*S*)-**7** followed by reduction of the formed aldehyde gave access to the furan-bearing compound. Although different fluoride sources were screened, deprotection of the TBS group was still low-yielding under optimized conditions using $Et_3N{\cdot}3HF$ in THF. In a different approach, we fully oxidized the A-ring prior to modification of the side chain: deprotection of the TBS group of (11*S*)-**7** using HF in MeCN/THF was followed by DMP-mediated oxidation of the secondary alcohol to install the carbonyl moiety at C-3 so that **10** was obtained with 89 % yield. Unfortunately, dehydrogenation at C-1/C-2 (*e.g.* DBU, O_2, CH_2Cl_2) was low-yielding and the isolated diene dione was found to be very unstable and unsuitable for the ensuing steps of the synthesis. We therefore decided to use the approach outlined in Scheme 2: after installation of the carbonyl moiety at C-3, oxidative cleavage of the terminal double bond furnished aldehyde **11** in 76 % yield. Having this tricarbonyl-bearing substrate **11** in hand, we addressed the selective reduction of the aldehyde at C-12 without reducing the keto groups at C-3 or C-8. Upon screening several reducing agents (table S1 in the Supporting Information), we found that the following conditions work very well: addition of three equivalents of $NaB(OAc)_3H$ to a solution of the aldehyde **10** in CH_2Cl_2 gave 63 % of the desired mono-reduced compound **12**.[12]

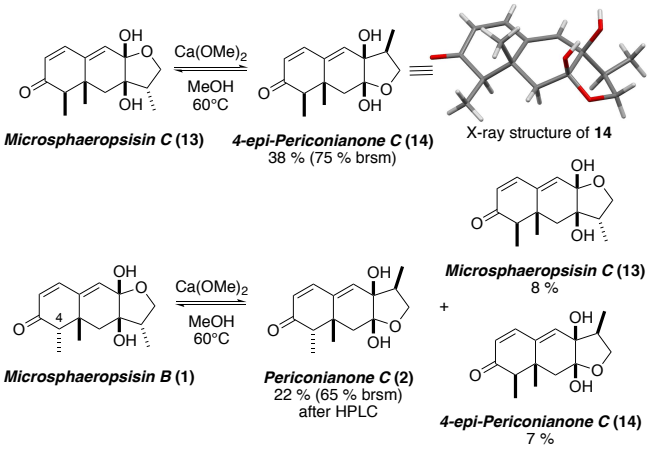
With intermediate **12** in hand, we started to investigate the final dehydrogenation step to microsphaeropsisin B (**1**). When the aldol reaction for construction of the C-ring in the total synthesis of periconianone A had been carried out in the presence of diazabicycloundecene (DBU) or the guanidine base triazabicyclodecene (TBD), this reaction had been accompanied by oxidation of the C-1–C-2 bond of the Bn- or PMB-protected (C-7–O–PG) tricarbonyl compounds to give the desired diene dione function prior to cyclization.[13] We applied the same reaction conditions to substrate **12** with the aim of not only dehydrogenating the C-1–C-2 bond, but also epimerizing the stereogenic center at the α-position to the carbonyl moiety at C-4 to form both microsphaeropsisin B (**1**) and C (**13**). Initial attempts by using DBU in CH_2Cl_2 only led to trace amounts of the desired compounds. Significantly higher yields of both natural products were achieved by applying TBD as base. After work-up, analysis of the ^1H NMR spectra of the crude reaction mixture still indicated the presence of unidentified side products. Therefore, we monitored this transformation by means of ^1H NMR spectroscopy: within 15 minutes, ^1H NMR signals indicating the formation of an unknown intermediate were observed, which disappeared with prolonged reaction time. Only traces of this intermediate were detected after four hours, which prompted us to extend the reaction time from 30 minutes to five hours. This modification not only gave better yields, it also proved to be the key for the successful isolation of pure microsphaeropsisin B (**1**), after attempts to separate this intermediate from microsphaeropsisin B had been unsuccessful using column chromatography or RP-HPLC.



Scheme 2. Synthesis of the natural products microsphaeropsisin B (**1**) and C (**13**). TBD: triazabicyclodecene, DMP: Dess–Martin periodinane.

Mechanistically, we suggest the intermediacy of a peroxide species in this transformation, which forms the B (**1**) and C (**13**) isomers after elimination of H_2O_2. This assumption is based on literature precedents that describe transformations of steroidal ketones into their corresponding hydroperoxy ketones in the presence of oxygen under basic conditions, and the isolation and characterization of such intermediates.[14] In order to examine if the dehydrogenation of **12** to **1** and **13** might proceed *via* a similar pathway, we measured the peroxide concentration of the reaction mixture: the presence of H_2O_2 was indeed indicated both after 15 minutes and 2.5 hours, and increased with prolonged reaction time. In order to rule out the oxidation of THF in this reaction mixture, we performed a test reaction in the absence of substrate **12**: after neither 15 minutes nor 2.5 hours could peroxide formation be detected in pure THF, confirming that the substrate is involved in this process. After separation by preparative HPLC, we obtained microsphaeropsisin C in 35 % yield, and microsphaeropsisin B in 10 % yield. The ^{1}H and ^{13}C NMR spectra of the synthetic compounds were in agreement with those reported for the isolated natural products (table S2, S3 and S4 in the Supporting Information).[6] However, we identified additional minor signals in the ^{1}H and ^{13}C NMR spectra. These signals are barely visible in the ^{1}H NMR spectra of the isolated compounds and no structural assignment had been performed by Zhang *et al*. After structural elucidation using 2D NMR spectroscopy, we could assign those minor signals to the open forms of the corresponding natural products, which are in equilibrium with their furan-type isomers (Scheme 2). Optical rotation values of both synthesized natural products were measured and compared with those reported in the literature for the isolated material in order to confirm that their absolute configurations are identical: the measured value of $[\alpha]_D^{25}$ = –20.2° (c = 0.60, MeOH) for **1** was in agreement with that reported in the literature ($[\alpha]_D^{20}$ = –16.0°, c = 0.10, MeOH); however, a significant discrepancy was observed for **13** with an optical rotation value of $[\alpha]_D^{24}$ = +67.0° (c = 0.48, MeOH) for the synthesized material and a reported value of $[\alpha]_D^{20}$ = –124.0° (c = 0.025, MeOH) for the isolated material. In order to establish the identity of the isolated natural and synthetic **13**, we measured a CD spectrum of synthesized microsphaeropsisin C. In contrast to the $[\alpha]_D$ value, this CD spectrum was in full agreement with that reported in the literature (see the Supporting Information). Microsphaeropsisin B (**1**) had already been isolated in 2009 from the endophytic fungi *Botryosphaeria rhodina* PSU-M35 by the group of Rukachaisirikul, named botryosphaerihydrofuran and assigned an incorrect structure.[15] Recently, Kutateladze and collaborators revised this structure based on computational predictions of its ^{13}C NMR spectrum and comparison with the reported spectrum for microsphaeropsisin.[16] With our measured ^{1}H and ^{13}C NMR spectra of synthetic microsphaeropsisin B (**1**) in $CDCl_3$ matching the one reported for natural botryosphaeri-hydrofuran, we provide additional supporting evidence to the structural revision by Kutateladze.[16]

With microsphaeropsisin B and C in hand, the stage was set to investigate the *furan shuffling hypothesis via* an α-ketol rearrangement for the transition of both natural products into their respective rearranged C-8–C-11 connected regioisomers. Our studies commenced with the transposition of microsphaeropsisin C (**13**). After adjusting the reaction conditions that had successfully triggered the reaction of **9** to **7** ($Ca(OMe)_2$, MeOH, rt) by heating to 60°C, we successfully brought about the conversion of **13** to a new compound, identified as the 4-*epi*-isomer (**14**) of periconianone C (**2**) after isolation and structural elucidation by single-crystal X-ray analysis.[17] Analysis of the reaction mixture by ^{1}H NMR spectroscopy identified the product **14** and starting material **13** with hardly any side products. After purification by flash column chromatography, 4-*epi*-periconianone C (**14**) and microsphaeropsisin C (**13**) were isolated in almost equal quantities.



Scheme 3. Synthesis of the natural product periconianone C (**2**) and its 4-*epi*-isomer **14** *via* a proposed biogenetic α-ketol rearrangement.

The same reaction conditions ($Ca(OMe)_2$, MeOH, 60°C) were applied to microsphaeropsisin B (**1**) with the aim of triggering the α-ketol rearrangement to periconianone C (**2**). The desired natural product **2** was observed along with remaining starting

material **1** by ^{1}H NMR analysis of the crude mixture (Scheme 3). Additionally, during the course of the reaction, the basic conditions resulted in partial epimerization at C-4 to form microsphaeropsisin C (**13**) as well as 4-*epi*-periconianone C (**14**). Separation of the crude reaction mixture containing the four known isomers was found to be challenging: using column chromatography, we were only able to separate the more apolar 4-*epi*-periconianone C from the other three compounds **1**, **2** and **13**. A reasonable separation for microsphaeropsisin B (43 %) and C (8 %) as well as for periconianone C (22 %) and 4-*epi*-periconianone C (7 %) was achieved by preparative HPLC (see the Supporting Information). The ^{1}H and ^{13}C NMR spectra of synthetic periconianone C were in agreement with the spectra reported in the literature for the isolated material, and both the opened and closed structures of **2** were observed in the ^{1}H NMR spectrum. The optical rotation values for synthetic ($[\alpha]_D^{22}$ = –53.8°, c = 0.19, MeOH) and natural material ($[\alpha]_D^{22}$ = –45.5°, c = 0.11, MeOH)[7] do not differ significantly.

In conclusion, the first enantioselective total syntheses of microsphaeropsisin B (**2**) and C (**13**) in 15 steps from known γ-hydroxy carvone are presented. The tandem OH-insertion/[3,3] rearrangement as well as the α-ketol rearrangement have been incorporated successfully into our synthetic route for the synthesis of the desired (11*S*)-isomer of α-allylated α-hydroxyenone **7**. For the last five synthetic transformations, selective reduction of the aldehyde in the presence of two other carbonyl moieties was identified as an exigent challenge. After careful screening of reaction conditions, we achieved the desired mono-reduction with high selectivity and without over-reduction of the substrate. Unusual autoxidation conditions using a guanidine base and molecular oxygen not only lead to dehydrogenation of the C1–C2 bond to form the desired diene dione, but also to epimerization of the C-4 position, thereby forming both C-7–C-11-connected natural product stereoisomers. By heating the substrates with calcium methoxide in MeOH in a slight variation of our previously reported protocol for the α-ketol rearrangement, we were successful in observing the rearrangement of both natural products microsphaeropsisin B and C into their corresponding regioisomers periconianone C and 4-*epi*-periconianone C. These findings provide strong experimental evidence for the biogenetic hypothesis of microsphaeropsisin B being a biosynthetic intermediate to periconianone C and hint at the occurrence of other eremophilane-type terpenoids bearing a C-8–C-11 linkage.

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Keywords: terpenes • natural products • total synthesis • rearrangements • biogenesis

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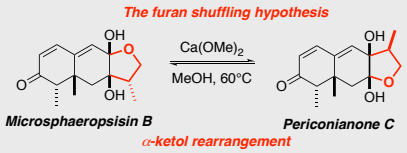
[17] CCDC-1896118 contains the supplementary crystallographic data for compound **14**. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures.

COMMUNICATION



An unusual furan rearrangement to biogenetically connect the eremophilane natural product microsphaeropsisin B and its regioisomer periconianone C is postulated. Strong experimental support for this intriguingly simple mechanistic pattern is provided by total chemical synthesis comprising an α-ketol rearrangement.

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